Supplemental Figure 4
Serum CK19 ELISA test

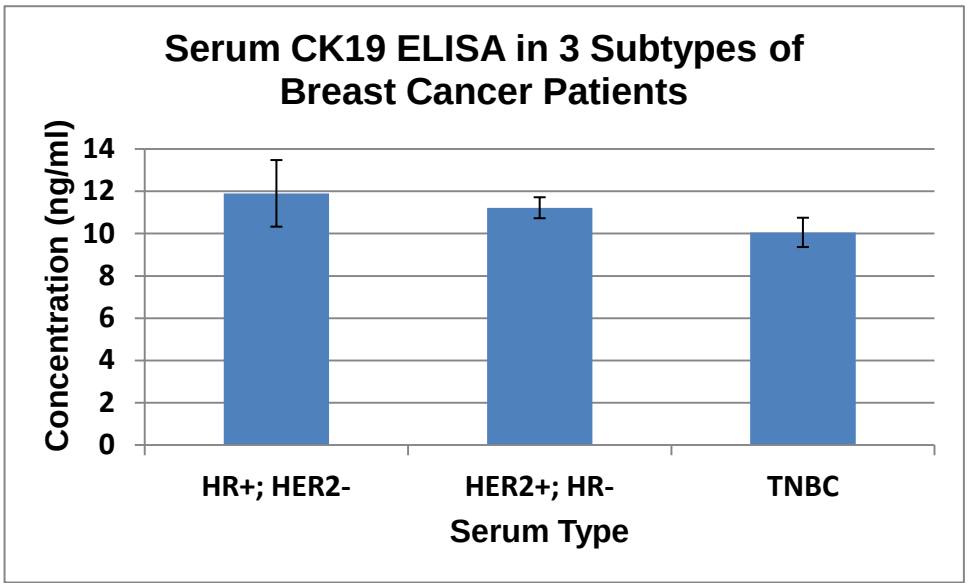
Method:

Blood samples were collected from advanced breast cancer patients who enrolled in an IRB approved clinical trial. After placing blood at room temperature for 15 minutes, blood was centrifuged at 2,500 rpm at 4º C for 10 minutes, aliquot and stored at -80º C until test. Concentrations of serum CK19 were detected and quantified using Human Cytokeratin 19 ELISA Kit according to the manufacture's instruction (BIOTANG Inc, Cat#. HU9721). 10 serum samples of each subtype of breast cancer patients (TNBC, HER2+/HR- and HER2-/HR+) were tested and 50ul of each serum was used. All samples were tested in duplicate.

Results and Discussion:

The concentration of serum CK 19 in TNBC patient is lower compared with other two subtypes of breast cancer patients. Although the data is consistent with the mass spectrometry and Western blot analysis of the breast cell line media, due the small tested sample size, the differences among the three groups were not significant. In future studies we will further test the validity of these potential biomarkers in serum from a representative panel of different breast cancer patients by using selected ion monitoring (SIM) in a Q Exactive mass spectrometer. The benefits of SIM analysis is that we can preprogram the mass spectrometer to only identify and quantify a panel of 100 or more peptide ions corresponding to a panel of potential biomarkers in each breast cancer patient serum sample.

Sample	Mean Concentration (ng/ml)	Std Dev	Std Err Mean
HR+; HER2-	11.8989	4.978962	1.5749
HER2+; HR-	11.219	1.567818	0.4958
TNBC	10.0531	2.191402	0.693


Serum CK19 ELISA in 3 Subtypes of Breast Cancer Patients
Concentration (ng/ml)
14
12
10
8
6
4
2
0
HR+; HER2-
HER2+; HR-
TNBC
Serum Type